

Biofuel Production from *E.Coli*: A Review

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Abstract

One of the major questions of the century is how to tackle the problem of pollution. There is not a single individual that has been left untouched with pollution. Our country is among top 10 most polluted countries. One of the solutions of this problem is the production and use of biofuels. Biofuels not only have a high calorific value but also are a great help to reduce the impact of pollution in our environment. A major breakthrough to tackle the problem of pollution is the use of microbes; it not only solves our problem but also adds value to our environment. This review presents a general review on the production of Biofuels from *E.Coli* – commonly found bacteria. The production of biofuel from *E.Coli* is a good example how we can use the bacteria for our good will. The production of biofuel from bacteria counteracts the thinking of a major class of people who think that bacteria only cause harm to the people. Let us know how *E.Coli* can be used to overcome the problem of the century.

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1. Introduction

Seeing the Present economy and pollution factor now the time has come when we need to think something beyond the use of petroleum fuel. We need to focus on the use and production of Biofuels. Biofuels are the fuels that are produced either plants or from living organisms such as Yeast or *E.coli*. [8] Petroleum fuels are limited in our blue planet and their use in such a great speed can lead to their end in the upcoming time. Further the use of petroleum fuel causes a lot of pollution and harm to our environment. Biofuels on the other hand are ecofriendly and can be artificially produced in the lab. Biofuels are a great tool to tackle the upcoming challenges of the storage of petroleum based fuels. The most advanced research that is being carried out all over the world in the production of biofuels from microbes such as Yeast and *E.coli*.

Our challenge is to increase the yield before we can go into any form of industrial production according to Prof John Love, University of Exeter (April 22, 2013) who has a great contribution in the production of biofuel from *E.coli*.

1.1 Why only Bacteria are used for the production of Biofuels?

Biofuels can be produced from various plants such as Corn, Sugarcane, palm oil, Jatropha etc. But the major problem with the production of Biofuels from plants is their existence in a limited amount and a limited life span. Production of large amount of Biofuels from these plants needs a lot of plants and to sustain such a large population it becomes nearly impossible to produce such a huge amount of Biofuel from plants [2]. So the best alternative is the production of Biofuels from microbes. Microbes are present in huge amount in our atmosphere and the major profit with the production of Biofuel from microbes is that this process is ecofriendly, further microbes around us are

easily available and there is no point that they will get exhausted in future.

Researchers are going on all over the world for the production of biofuels through various processes.

Recently Isopropanol and Butanol have been produced from *E.coli* the production of Isopropanol and Butanol is a two-step process where in the first step sugar produces fatty acid and second step is conversion of corresponding fatty acid to Biofuel. *E.coli* converts Butyric acid to alcohol. *E.coli* by use of amino acid Bio pathway generates 2- Keto acid precursors that produce alcohol by single heterogeneous reaction [21].

At normal conditions and controlled conditions *E.coli* produces 60mm Butanol in 24 hours that until 240 hour produces 7.6 m. mol/l/hour Butanol. At time the pharmaceutical industry reacted a way by which *E.coli* could excrete fuel first after it had been modified but the major problem with it was that just after the production of fuel the fuel sticks around the bacteria killed the bacteria. But through the process of genetic manipulation of *E.coli* the bacteria can produce fuel more efficiently without the need to be replaced.

Another way of producing Biofuel is through cellobionic acid study has shown that *E.Coli* is able to produce & biofuel using cellobionic acid as a carbon source. *ascB* gene on *E.Coli* is responsible for producing Biofuel from cellobionic acid. Along with it *ascB* stream can be complemented by cellobionic acid Phosphorylase from *Neurospora*. Another stream of *E.Coli* was engineered and it expressed the Isobutanol production pathway and as a result cellobionic acid was converted to isobutanol.

With advancement in the field of genetics *E.Coli* can be engineered to convert enojed glycerol to ethanol by the process of fermentation. This process has a dual positive impact because glycerol is the major waste product in the process of biodiesel production and this glycerol is further used for bioethanol productive with the help of *E.Coli*. The *E.coli* grows on glycerol and ethanol is produced. This strain of *E.coli* grows on glycerol and during the process of fermentation large amount of ethanol is produced. It has also been found that the growth of *E.coli* strain is fastest at O₂ abundant conditions. [1, 3, 6]

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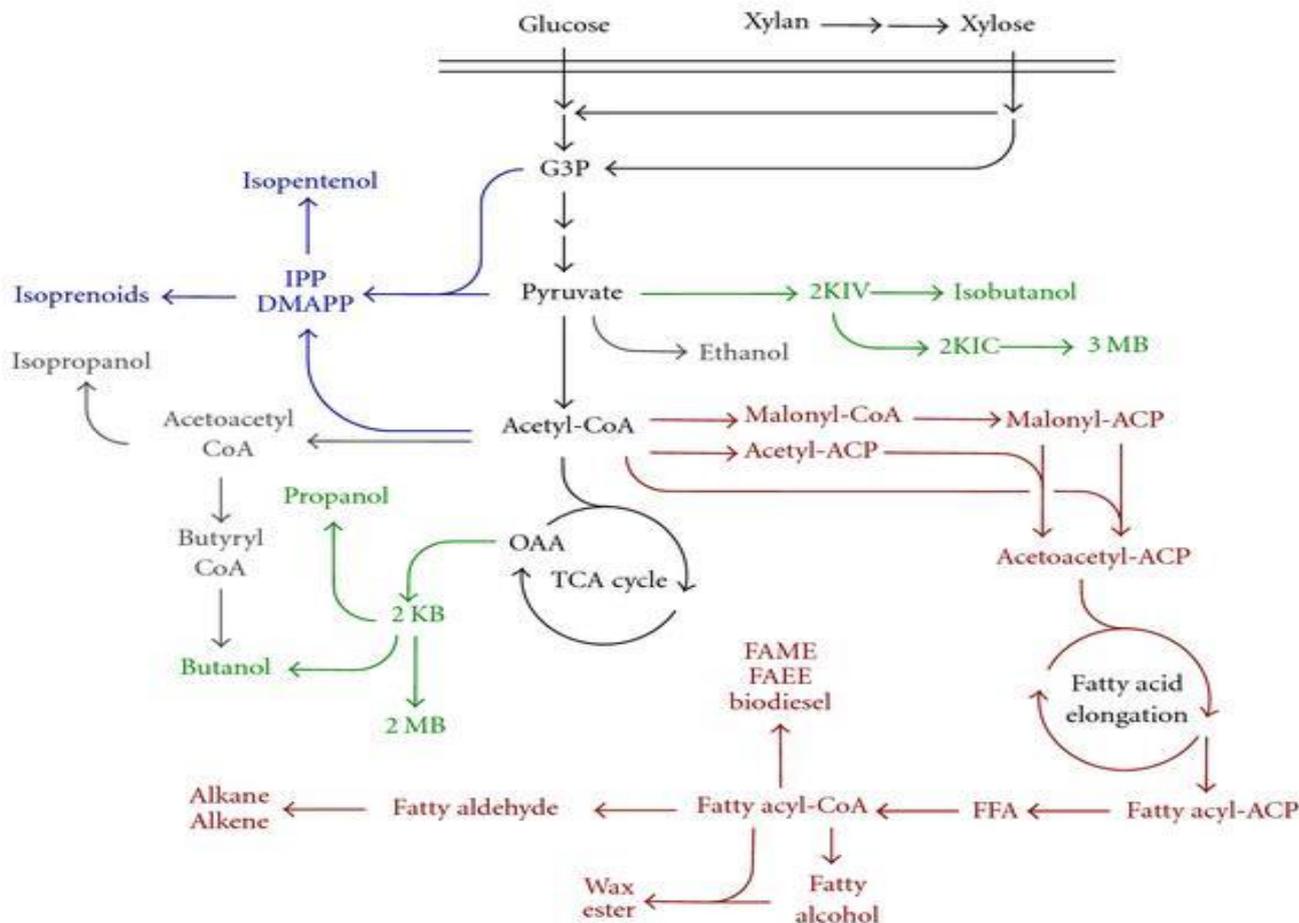


Fig. 1 Cycle showing conversion

E. coli grows on glycerol and ethanol is produced. This strain of *E. coli* grows on glycerol and during the process of fermentation large amount of ethanol is produced. It has also been found that the growth of *E. coli* strain is fastest at O₂ abundant conditions. [1, 3, 6]. *E. coli* also produces Biofuel by accomplishment of feat by the mixture and matching of different bacteria that have been inserted to *E. coli* (gives of bacteria are inserted to *E. coli*). These gives inserted code for various enzymes and this result in the production of Biofuel in form of Butanol.

We know that traditional Biofuels mainly comprise of fatty acid that have been derived from plant oil. Scientists at J.B.I (Joint Bioenergy Institute) have engineered *E. coli* to produce biofuels. Due to change in molecular linkage and bond structure these Biofuels have very good combustion qualities [23].

The use of Petroleum fuel cause a lot of pollution but the production of commercial Biofuel would lead to the decrease in Pollution level by 80% by 2050. *E. coli* has the capability to turn sugar into fat to build cell membrane synthetic fuel oil molecules are created by a natural oil process production [14,20, 30].

Various series of Biochemical reactions allow production of enzymes that allow various types of cells to convert

various raw materials to substances that are necessary for cell to survive and produces Biofuel so scientist have created a mutant *E. coli* that is capable of producing Biodiesel[20]. The main advantage of using *E. coli* is due to its capability to synthesize various mass synthesizing products through microbial process. Through this process Biofuel is produced. All this takes place through Recombinant DNA technology where Recombinant DNA is joined to foreign DNA that are further inserted to *E. coli* bacteria that produce new combination of plasmids for the production of Biofuel[26] The most widely used genome of *E. coli* is K-12 strain that occurs in nature. This stream has been sequenced for Biofuel Production.

2. Some other microbes used in Biofuel Production

2.1 *Chlorella Sp*[20] – 30-L-24-96, 30-L-24-85, 30-4, 24, 43 A119 Logos, A118waco, 3N4129 (4), L-729, 3N8/13 (A), MIP2 30-4-24, 102, Syngen 2-3, NC64A, 47EX26, 30-L-24-86, *Zymomonas mobilis* – ZM4

2.2 *Cryptocodium cohnii* [19] – SM1, MBS2, SG3, 01S1, M7, GP1, M1, PS1, Puerto Rics, GCc, GCS, I2, Gv, RHP1, BBSr1, Da1, H3, BaD1, Ab1, SJ, Kws1, Y6, LPP1, NP4, L4, GCP, MB3, TR3, 7010

2.3 *Chlorella sp-* [19]

Saccharomyces Cerveaceae

2.4 *Chlorella Vulgaris* [10] –

E.coli – TOP 10, ADP1, K-12, MG16SS, K12W3110, 45/41, BL21, PXL72, A1CC9637, A7CC11303, AICE15244

2.5 Clostridium Acetobutylicum – A7CC824

3. Best temperature for the production of Biofuels[31]

Various researches show that at 370C production of fatty acid ethyl esters was negligible at 300C 922mg/l-1 FAEE were produced while at 250C 652 mg/l-1 FAEE were produced.

The most optimum temperature for the production of Biofuels is 30°C - 37°C.

At 46°C *E.Coli* dies.

3.1 Best pH. For Biofuel production

Most of the researches show that optimum growth of *E.Coli* and ethanol production is at pH 6.0-6.5. [34] The highest value of ethanol production by *E.Coli* on growth curve was found to be at 7.0 pH. [34]

3.2 Various carbon sources for Biofuel production by *E.Coli*. [14]

Xylose, glucose, glycerol, corn liver hydrolysate, dextrin, galactose, maltose, sucrose, fructose, starch, cellulose, lactose, mannose.

3.3 Various Nitrogen Sources: - [16, 14]

Ammonium chloride, Ammonium Sulphate, Ammonium Nitrate, Yeast extract, meat extract, Leptone, Tryptone.

3.4 Ethanol yield of various *E.coli* streams with glucose as carbon content [23]–

Strain	Genotype	Ethanol yield (g/g)	Ethanol yield %	Reference
KO3	<i>pfl</i> ⁺ <i>pfl</i> : <i>(pdc</i> ⁺ <i>adhB</i> ⁺ Cm ^R)	0.13 g/g glucose	26	Ohta et al. (1991)
KO4	KO3, selected for high Cm ^R	0.56 g/g glucose	> 100 ^a	Ohta et al. (1991)
KO11	KO4 <i>frd</i>	0.54 g/g glucose	> 100 ^a	Ohta et al. (1991)
KO11	KO4 <i>frd</i>	0.46 g/g xylose	89	Yomano et al. (1998)
LY01	KO11, selected for high ethanol tolerance	0.44 g/g glucose	85	Yomano et al. (1998)
LY01	KO11, selected for high ethanol tolerance	0.47 g/g xylose	92	Yomano et al. (1998)
LY 160	KO11, Δ <i>frd</i> : <i>celY</i> _{Ec} Δ <i>adhE</i> Δ <i>ldhA</i> Δ <i>ackA</i> <i>lacA</i> : <i>casAB</i> _{Ko} <i>rrlE</i> : <i>(pdc</i> _{Zm} - <i>adhA</i> _{Zm} - <i>adhB</i> _{Zm} -FRT- <i>rrlE</i>) <i>pflB</i> ⁺	0.49 g/g xylose	95	Yomano et al. (2008)
AH003	KO11, Δ <i>ldhA</i> Δ <i>pflA</i>	0.54 g/g glucose	> 100 ^a	Hildebrand et al. (2013)
AH003	KO11, Δ <i>ldhA</i> Δ <i>pflA</i>	0.35 g/g gluconate	98	Hildebrand et al. (2013)
SZ420	Δ <i>frdBC</i> Δ <i>ldhA</i> , Δ <i>ackA</i> Δ <i>focA-pflB</i> Δ <i>pdhR</i> : <i>pflBp6-pflBrbs-aceEF-lpd</i>	0.45 g/g glucose	90	Zhou et al. (2008)
TCS083	Δ <i>zwf</i> Δ <i>ndh</i> Δ <i>scfA</i> Δ <i>maeB</i> Δ <i>poxB</i> Δ <i>ldhA</i> Δ <i>frdA</i> Δ <i>pta</i> :Kan ⁻ , carrying plasmid with <i>pdc</i> _{Zm} and <i>adhB</i> _{Zm}	0.48 g/g glucose	94	Trinh et al. (2008)
TCS083	Δ <i>zwf</i> Δ <i>ndh</i> Δ <i>scfA</i> Δ <i>maeB</i> Δ <i>poxB</i> Δ <i>ldhA</i> Δ <i>frdA</i> Δ <i>pta</i> :Kan ⁻ , carrying plasmid with <i>pdc</i> _{Zm} and <i>adhB</i> _{Zm}	0.49 g/g xylose	96	Trinh et al. (2008)
TCS099	Δ <i>zwf</i> Δ <i>ndh</i> Δ <i>scfA</i> Δ <i>maeB</i> Δ <i>poxB</i> Δ <i>ldhA</i> Δ <i>frdA</i> Δ <i>pta</i> Δ <i>mdh</i> :Kan, carrying plasmid with <i>pdc</i> _{Zm} and <i>adhB</i> _{Zm}	0.37 g/g glycerol	74	Trinh and Sreenc (2009)
TCS099	Δ <i>zwf</i> Δ <i>ndh</i> Δ <i>scfA</i> Δ <i>maeB</i> Δ <i>poxB</i> Δ <i>ldhA</i> Δ <i>frdA</i> Δ <i>pta</i> Δ <i>mdh</i> :Kan, carrying plasmid with <i>pdc</i> _{Zm} and <i>adhB</i> _{Zm} evolved with metabolic evolution in glycerol/tetracycline	0.49 g/g glycerol	98	Trinh and Sreenc (2009)

^aHigher ethanol titers were reached due to ethanol production from the catabolism of complex nutrients. The yield was calculated on initially added sugar.

4. Conclusions

It has become apparent that the biofuels derived from crops are not sufficient to satisfy our needs. Future needs of the demands of energy. A lot of research of being carried out for the production of next generation biofuels. The main focus of this researches is synthesis of microbial fatty acids. These fatty acids and their derivatives are a future substitution of petroleum fuels. It has been found that *E.coli* is the best microbe for biofuel production. *E.coli* in the past time has proved to be a little bacterial engine. Researches from all over the world have created mutant *E.coli* that is capable of producing biofuel. The production of biofuel from *E.coli* has the potential to lower the greenhouse emissions and reduce the cost for a cleaner environment. Biofuel production from *E.coli* has emerged as a source of sustainable energy as it is one of the first scientific endeavors in which researchers have manipulated biological systems.

The advantage of using *E.coli* for biofuel production includes the capability of *E.coli* to grow quickly.

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